Is Prevention Possible?

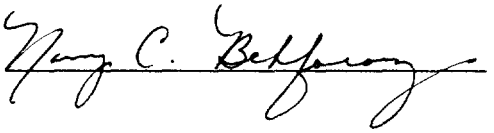
The Search for a Vaccine for the HIV Virus

An Honors Thesis (HONRS 499)

by

Daniel E. Boedeker

Dr. Nancy Behforouz



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ABSTRACT:

Acquired Immune Deficiency Syndrome (AIDS) has been the focus of vaccinologists since the virus that causes the disease was first described in 1984. With all of AIDS unique characteristics, the virus to date has evaded the successful development of a preventative vaccine. This thesis addresses the problems associated with an AIDS vaccine being developed, current research that has been sought as a cure to the disease, and what steps the potential vaccines have to undergo before they are available for human usage.

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Is Prevention Possible?

The Search for a Vaccine for the HIV Virus

Since the HIV epidemic began in the 1980s, it is estimated that more than 28 million people have been infected with HIV. Of that 28 million, there are approximately 22 million people currently living with HIV and 3 million new infections being added to the total each year worldwide. Ninety percent of the 28 million global infections have occurred in developing countries, with 65 percent in countries of sub-Saharan Africa and another 20 percent in countries of South and Southeast Asia (Heyward et al, 1998). Over the past decade and a half, the HIV epidemic has progressed virtually unimpeded to affect nearly every country on every continent in the world (Heyward et al, 1998).

Since 1984 when the Human Immunodeficiency Virus (HIV) was singled out as the causative agent in Acquired Immune Deficiency Syndrome, preventative measures have been sought by medical researchers in hope that in ten to fifteen years from now, a vaccine will be discovered that can eliminate this deadly disease from the earth. AIDS is caused by a DNA retrovirus and can be passed on from infected to uninfected individuals through four main modes of

transport: blood, semen, vaginal fluid, and breast milk. Its mode of operation is to target and infect the CD 4+ cells of the immune system which are vital to the immune responses that our body use to eliminate diseases caused by microbial agents.

Entering U.S. society in the early 1980s first in the gay and intravenous drug using populations, AIDS has put fear into countless numbers of people because there presently is not a cure for the disease, and once it develops following HIV infection the chances of long term survival are minimal. Although we actually know how to prevent HIV infection through effective prevention programs of education, HIV testing and counseling, condom promotion, serological screening of donated blood, needle exchange, and other interventions, the HIV epidemic continues, and, in some areas of the world such as Southeast Asia, Eastern Europe, and Russia, it is expanding exponentially (Heyward et al, 1998). Since the virus infects the immune system, the use of drug therapy can only help to lengthen the amount of time it takes the disease to overwhelm and debilitate the immune system (Des Jarlais et al 1997). There is no question that a safe, effective HIV preventive vaccine is urgently needed to bring the HIV/AIDS epidemic under control, and it is our hope and belief that modern

medical technology will be able to overcome this disease, as it has for so many countless others.

Vaccinology

Scientists in the past have been able to find preventative vaccines for the use of the general population since cowpox was first used to vaccinate for the human disease of small pox. From numerous achievements, a science devoted solely to the development of vaccines has been founded. Vaccinology, the engineering and development of vaccines to prevent infectious diseases, has been challenged for a vaccine which protects against AIDS because it is so unique in its mode of infection, being a primarily sexually transmitted disease, and the role it takes in the immune system once it has infected an individual (Hilleman, 1998).

Though misguided in its first decade, current vaccine research is directed to use any and all viral antigens and to elicit both cell-mediated and antibody immune responses, with memory at the mucosal sites of the body, which is the primary portal of entry (Hilleman, 1998).

Problems Associated with the Development of an Effective HIV Vaccine

Although dozens of potential vaccines have been under development ever since HIV was discovered in 1984, most

have reached a dead end in the laboratory and none has yet reached the phase III stage where it can be widely tested to see if it is effective (Perlman, 1998).

The development of a safe and effective vaccine for the prevention of AIDS has thus far proven to be extremely difficult, at least in part due to complexities associated with HIV-1 and its pathogenesis (Graham and Wright, 1995). Unlike some other viruses, HIV can be transmitted and can exist in the body not only as free virus but also within infected cells, sometimes for long periods of time in a latent provirus. Another factor complicates any attempt to provide protection from HIV infection. According to the World Health Organization (WHO), 80 percent of all HIV transmission worldwide occurs sexually. Thus, to be effective, an HIV vaccine may need to stimulate mucosal immunity. Mucosal immune cells that line the respiratory, digestive and reproductive tracts and those found in nearby lymph nodes are the first line of defense against infectious organisms. Unfortunately, relatively little is known about how the mucosal immune system protects against viral infection (Johnston, 1997).

Perhaps the most difficult challenge for vaccine researchers is that the major target of HIV is the immune system itself. HIV infects the key CD4+ T cells that

regulate the immune response, modifying or destroying their ability to function (Johnston, 1997). After infection into the host cell, the RNA of HIV is reverse transcribed into DNA and incorporates its genetic material in the host cell chromosome (Behforouz, 1999). If the cell reproduces itself, each new cell also contains the HIV genes. There the virus can hide its genetic material for prolonged periods of time until the cell is activated and makes new viruses. During a clinical dormancy period, the disease can still be transferred to other individuals although the carrier may appear to be clinically asymptomatic.

Understanding how HIV disease evolves, especially during early infection is a high priority of AIDS researchers (Johnston, 1997). Scientists have shown that no true period of biological latency exists in HIV infection. After entering the body, the virus rapidly disseminates, homing to the lymph nodes and related organs where it replicates and accumulates in large quantities (Johnston, 1997). The filtering system in these lymphoid organs, which are so effective at trapping pathogens and initiating an immune response, may help destroy the immune system because HIV infects the steady stream of CD4+ T cells that travel to the lymph organs in response to HIV infection (Johnston, 1997).

The genetic variability of HIV-1 strains has also been correlated with *in vitro* growth characteristics of the virus, some of which may be relevant for vaccine development. Some strains referred to as 'slow/low', replicate slowly and to low titer in T-cell lines, and to induce syncytia (SI) {54-56}. Most viruses isolated from recently infected persons exhibit the slow/low phenotype, whereas viruses isolated from patients with more advanced disease are of the rapid/high phenotype. These findings suggest that HIV is usually transferred with a phenotype that doesn't replicate rapidly, but later evolves to the rapid/high phenotype once it has been in the body.

The recent description of individuals transiently infected with HIV-1, as well as persons who survived HIV-1 infection for more than 15 years, indicates the ability of the immune response of certain individuals to control HIV-1 infection (Hulskotte et al, 1998). Moreover, vaccination-challenge experiments in macaques infected with simian immunodeficiency virus (SIV) have shown that protection against infection or development of disease may be achieved in the absence of sterilizing immunity, suggesting that the goals for AIDS vaccine development may have to be refined (Hulskotte et al, 1998). This may suggest that the ultimate goal of HIV research may have to be directed to

the prevention of disease rather than immunity through infection (Behforouz, 1999). However, development of true immunity to this virus in humans has not been clearly demonstrated and immune correlates of protective immunity remain uncertain (Hulskotte et al, 1998).

One of the most unique properties of the AIDS virus that makes it so elusive to the vaccinologists is its ability to mutate while maintaining the unique properties that are so destructive to the human immune system (Mortara et al, 1998). With multiple genetic subtype of HIV-1 differing up to 30% of nucleotides in their envelope coding sequences, having been identified in the global epidemic, it is easy to see why thus far it has been so difficult to find a vaccine that is able to give immunity to such a large diversified group of envelope proteins (Mortara et al, 1998). In the United States alone, where HIV-1 infection with subtype B predominates, the interisolate diversity in the viral envelope is 15% or more (McCutchan et al, 1998). Although it is recognized that geographic, temporal, and demographic variables can affect the genetic diversity of HIV-1 strains, there have been few opportunities to evaluate these factors by population-based sampling, adding to the problems associated with the diversity seen in the virus.

Another major problem in developing any AIDS vaccine is finding a system that exploits two distinct aspects of the human immune system, said Dr. Jay Levy, the virologist at the University of California at San Francisco who was one of the discoverers of HIV (Perlman, 1998). One function of the immune system that a vaccine must trigger is to induce the body to release antibodies, specialized proteins that attack the virus as soon as it invades, Levy said. The other is to mobilize cells that can either kill the virus directly or prevent it from replicating after it has begun infecting -- even when the virus is latent and lying hidden within the tissues it has infected (Perlman, 1998).

Another problem in the development of an AIDS vaccine is that the disease is only in the human population, and while other animals may get immune deficiency syndromes, there is not a really good model of disease for HIV in animals (Behforouz, 1999). Chimps get infected when they are inoculated, but apparently they do not get sick (Behforouz, 1999).

Researchers trying to develop a vaccine also run into problems involving the long incubation period characteristic of HIV, so it takes a very long time, sometimes years to be able to see how effective the vaccine would be (Behforouz, 1999).

Cognizant of all of these problems, along with others not yet delineated, scientists have delved further into the study of the virus, so that one of many different approaches to coping with the disease may finally see full fruition in the development of a useful vaccine able to be utilized by the entire human population.

Different Approaches to the Development of an HIV Vaccine

In order to discover and promote an effective HIV vaccine researchers must keep certain criteria in mind. First the vaccine should give long-term immunity to the individual. In order to achieve this, accumulating evidence suggests that such a vaccine must efficiently elicit an HIV-1 specific cytotoxic T lymphocyte (CTL) response. Although humoral or antibody responses have helped in the past, scientists are rapidly coming to the conclusion that a CTL response must occur in order for long term vaccination to be possible (Letvin). The ideal HIV vaccine would be inexpensive, easy to store and administer and would elicit strong, appropriate immune responses that confer long-lasting protection against both bloodborne and mucosal (sexual) exposure to many HIV subtypes. These criteria will ensure that the entire population may take advantage of the new found technology, in hope that eventually the AIDS virus may have a similar destiny to

that of the small pox virus, which was eradicated from the face of the earth.

Live Attenuated Virus

Traditionally, the most effective viral vaccines have been derived from live-attenuated viruses (e.g. measles, mumps, rubella, oral polio, and yellow fever vaccines). A live attenuated vaccine based on a strain of Simian Immunodeficiency Virus (SIV) has long been considered the best hope for an AIDS vaccine. The virus would be weakened by the deletion of information from three genes thought to be necessary for causing the disease including the nef gene (Pantaleo et al, 1995). Indeed, scientists have identified a naturally occurring attenuated HIV strains that lacks HIV's nef gene (Pantaleo et al, 1995). Among six Australians with this nef-deleted HIV through blood transfusions received between 1981 and 1984, none has developed HIV disease (Pantaleo et al, 1995). These individuals with nature's form of the attenuated virus provide some information on the course of disease after infection, which may be helpful for the development of an effective attenuated vaccine.

When used as vaccines, such attenuated viruses can protect monkeys against subsequent infection with the same and, to a lesser extent, different disease-causing strains

of SIV. In the SIV model, varying the number or location of genetically engineered deletions in the SIV genome can alter the level of attenuation. Preliminary results suggest that protective efficacy may vary inversely with the level of attenuation: with more deletions the vaccine becomes safer, but less effective (Desrosiers, 1992). Live, attenuated SIV vaccines can induce a diverse and persistent immune response against SIV. Most likely, the protective efficacy seen in monkeys is immune-mediated, since levels of antibodies and cellular immune responses increase over time, and the longer one waits to challenge vaccinated monkeys with a pathogenic SIV, the better the protection (Bagarazzi et al, 1997). Theoretically, an attenuated virus can elicit strong persistent antibody and cellular immune responses, as seen with many other vaccines that follow this route of action because it closely resembles the intact virus (British Medical Journal 317). This approach has been successful when tested on macaque monkeys, protecting them from normally lethal doses of the full strength virus (British Medical Journal 317). The trade-off to this benefit however is the potential risk that the modified virus will maintain some degree of virulence or may mutate or recombine back to virulence and cause disease.

Biological companies all over the world are now in pursuit of developing a live attenuated virus in hopes that the results seen in the monkeys can be transferred to the human population. Therion Biologics of Cambridge, Massachusetts is one such company that is now developing a live attenuated vaccine, but the company shares many concerns about such vaccines being administered (British Medical Journal 317). They speculate that the weakened virus might cause AIDS in people with weakened immune systems or that the virus may revert into a more virulent form once it has already been administered to a patient (British Medical Journal 317).

A study out of the Department of Infectious Disease and Microbiology at the University of Pittsburgh has developed a live attenuated vaccine, with a replication-defective HIV pseudotype with vesicular stomatitis virus G protein (VSV-G) (Tung et al, 1998). This pseudotyped HIV can infect many cell types, including human and simian cells, and undergoes only one round of replication (Tung et al, 1998). Furthermore, antibody immune response can be detected in mice immunized with VSV-G-pseudotyped replication-defective HIV (Tung et al, 1998). This novel approach to the live-attenuated version of the HIV vaccine may prove to be very effective at producing an immune

response in the body, while minimizing the potential for the virus to revert back into a more virulent form.

Although there have been advances and triumphs in the development of a live attenuated virus, in 1995, Dr. Ruth Ruprecht of the Dana Farber Cancer Institute in Boston found that the weakened virus eventually triggered the simian version of AIDS when administered to baby monkeys. The new findings, reported at the 12th world AIDS conference in Geneva by Dr. Ruprecht, showed that in time the vaccine also cause AIDS in adult monkeys: "What we saw in infants is a fast forward version of what could happen in adults with an attenuated vaccine" (British Medical Journal 317).

Despite these clear setbacks made obvious by the latest disconcerting findings, a group of about 300 doctors who had volunteered to be the first human to receive the attenuated virus plan to press ahead. "I'd take it tomorrow, and the others would, too," said Dr. Charles Farthing an AIDS specialist based in Los Angeles who is writing a plan for the clinical trial that will be submitted to the US Food and Drug Administration (FDA) (British Medical Journal 317). Despite the setbacks with the monkeys, Dr. Farthing believes that testing with an attenuated virus will begin within two years.

With the obvious discrepancies of results from various researchers, the encouraging finding must be balanced against several potentially serious risks which include 1) a very small fraction of individuals receiving other attenuated viral vaccines do develop disease, (Osborn, 1995) 2) an attenuated AIDS vaccine may still cause disease in some individuals (Osborn, 1995), 3) an attenuated HIV might mutate, recombine, or revert to a virulent form (Osborn, 1995) 4) other viruses used to make live, attenuated vaccines are cleared from the body,(with the exception of *Varicella* vaccine which is apparently not cleared)(Behforouz, 1999) 5) HIV and possibly the attenuated forms insert itself into the genes of the body's cells and remains there for life,(Osborn, 1995) 6)other retroviruses which integrate into an individual's DNA and are known to cause cancer in animals, (although HIV apparently does not lead to cancer)(Behforouz, 1999) 7) chronic HIV infection with an attenuated form might lead to the development of malignancies as well as other diseases apart from AIDS (Osborn, 1995).

Gp 120 Envelope Proteins

On the surface of the HIV virus, like many other viruses are envelope glycoproteins or antigens used for absorption to the surface of the cell. The gp 120 envelope

protein that is present on the HIV virion may prove to be an effective antigenic target for the development of a vaccine which could induce a humoral antibody response (Berman, 1998). It is the HIV-1 envelope glycoproteins that interact with receptors on the target cell and mediate virus entry by fusing the viral and cell membranes (Wyatt and Sodroski, 1998). It is possible to envision an antibody response to these glycoproteins, which could prevent infection particularly at mucosal surfaces by neutralizing the virus. The structure of the envelope glycoproteins has evolved to fulfill these functions while evading the neutralizing antibody responses of the immune system (Wyatt and Sodroski, 1998). A better understanding of the viral strategies for immune evasion should guide attempts to improve the immunogenicity of the HIV-1 envelope glycoproteins and, ultimately, aid in HIV-1 vaccine development (Berman, 1998).

Thus far, the gp120 subunit vaccines have been given the most attention, with some of the vaccines now being cleared to enter into various clinical trials. Eighteen participants in a phase I/II clinical trial were treated with recombinant gp 120 subunit-vaccines. Over the course of the trials the participants became infected with HIV-1 virus, and it was determined that only one of those who was

treated with the subunit-vaccines failed to develop a strong immunoglobin G response to the immunogen (Connor et al, 1998). However, the antibody response to rpg120 was transient, typically having a half-life of 40 to 60 days (Connor et al, 1998). The study further concluded that despite rigorous genetic analyses, using various breakdowns of data sets, no evidence that rgp120 vaccination exerted selective pressure on the infecting HIV-1 strains could be found (Connor et al, 1998). In summary, the study concluded that vaccination with rgp120 has had, to date no obvious beneficial or adverse effects on the individuals that that became infected during the course of the trials. (Connor et al, 1998).

Another study conducted by Lambert at the Johns Hopkins University in Baltimore researched the safety and immunogenicity of HIV recombinant envelope vaccines in HIV infected infants and children. Using Chiron rgp120 (SF-2) 15 or 50 micrograms; MicroGeneSys rgp160 (IIIB) 40 or 320 micrograms; Genetech rgp120 (MN) 75 or 300 micrograms; or adjuvant control (Alumin or MF059), children were randomized to a double-blind, placebo-controlled, dose-escalating study of vaccine administered intramuscularly at entry and 1,2,3,4, and 6 months later (Lambert et al, 1998). From the study, no adverse events were attributed

to the study vaccines (Lambert et al, 1998). Sixty-five percent of vaccinees but none of placebo recipients exhibited moderate or strong responses after enzyme immunoassay to HIV specific antigens (Lambert et al, 1998). The CD4 cell counts and subsequent quantitative HIV culture did not differ significantly among vaccine and control groups, nor were differences found among groups in HIV disease progression (Lambert et al, 1998). The study's final conclusion was that the rgp160 and gp120 subunit vaccines were safe and immunogenic in this population (Lambert et al, 1998).

With the progress seen in this approach to the HIV vaccine pursuit, the FDA has now granted permission for the first phase III tests of a vaccine based on this approach. Known as AIDSVAX, the vaccine is based on gp120 envelope glycoprotein. VaxGen, a subsidiary of Genentech, manufactures it (Josefson, 1998). The vaccine will be tested on 5000 US volunteers who have tested negative for HIV but are at high risk of contracting AIDS through sexual contact, and on 2500 volunteers from Thailand who are injecting drug users (Josefson, 1998). The phase III trials which are expected to last three years will compare vaccinated subjects with cohorts matched for age and risk factor, and the effect of the vaccine on AIDS prevention,

marker infections, and viral clearance will be studied. Although the FDA has approved the study, many scientists remain skeptical. In an interview with the *Wall Street Journal,* Dr. David Baltimore, the head of the NIH sponsored AIDS Vaccine Research Committee, said: "I personally believe that people should try what they want to try, but it's a very, very long shot to expect anything." Dr. Baltimore also raised the possibility that, when more effective vaccines are available, volunteers would be in short supply or ineligible because of participation in the AIDSVAX trial (Josefson, 1998).

Although the federal government stopped funding research on gp120 based vaccines in 1994, these earlier treatments were monovalent, using envelope protein of only one strain of HIV (Josefson, 1998). The new vaccines that are now being developed utilizing the gp120 glycoprotein are bivalent, incorporating the two most prevalent HIV envelope proteins (Berman, 1998). They will also be tailored to reflect the most prevalent strains in the populations they are given to (Josefson, 1998). With obvious setbacks in its past, the gp120 glycoprotein vaccine has seen a reemergence as a possible way to curb the growing HIV epidemic, but the most promising vaccine

possibility lies in using HIV own genetic code encompassed in it's DNA as a way to lead to it's ultimate demise.

DNA VACCINES

AT this point in the search for a vaccine for the HIV-1 virus type, DNA vaccine models have shown the most promise in being able to elicit immune reactions from both arms of the body's immune system, including humoral and cell-mediated immunity. A study by Ugen at the University of South Florida College of Medicine has shown that humoral and cellular immune responses have been produced, in mice, by intramuscular vaccination with DNA plasmids expressing HIV-1 genes, suggesting possible immunotherapeutic and prophylactic value for these constructs. The vaccination with these constructs decreased HIV-1 viral load in HIV-1 infected chimpanzees (Ugen et al, 1998). In addition to these findings, the study further showed that naïve (i.e. non HIV-1 infected) chimpanzees were protected against a heterologous challenge with HIV-1 (Ugen et al, 1998). Of course, HIV-1 does not replicate highly in chimps and they do not develop disease, so one must question whether this is a good study model or not (Behforouz, 1999). Ongoing phase I clinical trials show that therapeutic vaccinations indeed boost anti HIV-1 immune responses in humans. More

importantly, these constructs showed a good safety profile and also immunological potential (Ugen et al, 1998).

Other studies are now concentrating on improving the efficacy of the DNA vaccine, especially in regards to the HIV virus. A study out of the Max-von-Pettenkofer Institute in Munich Germany optimized codon usage of an injected DNA sequence to considerably increase both humoral and cellular immune responses. In the study, researchers generated a synthetic HIV-1 gp120 sequence, by replacing codons from the gp 120 sequence with genes expressed in humans (syngp120). In BALB/c mice, DNA immunization with syngp120 resulted in significantly increased antibody titers and cytotoxic T-lymphocyte reactivity, suggesting a direct correlation between expression levels and the immune response (Andre et al, 1998). More importantly, however, is that the syngp120 is characterized by rev-independent expression and a low risk of recombination with viral sequences (Andre et al, 1998). Rev is critical for transport of envelope mRNA to the cytoplasm for translation (Behforouz, 1999). Therefore, synthetic genes with optimized codon usage represent a novel strategy to increase the efficacy and safety of DNA vaccination against the HIV virus (Andre et al, 1998).

A study out of the Department of Bacteriology at the Yokohama City University School of Medicine in Yokohama Japan compared the immune responses seen when DNA vaccinations against HIV-1 were administered both intramuscularly and intranasally in mice. Although both routes of vaccination resulted in similar levels of cell-mediated immunity, the intestinal immunoglobin A response was higher following the intranasal immunization (Sasaki et al, 1998). This shows that the DNA vaccine has a good potential to be easily administered, not even making it necessary to inject into the muscle.

A recent chimpanzee experiment with a DNA candidate vaccine demonstrated protection on challenge with HIV after eight immunizations, including two booster doses of 1&181; g of DNA, over a one-year period. The first phase I trial of an HIV envelope DNA candidate vaccine is currently underway. Although this approach appears promising because of its ease of antigen modification, some safety issues still remain to be addressed, such as the unlikely possibility of integration or recombination of DNA with wild virus or a possible long-term carcinogenic effect (Heyward et al, 1998).

Inactivated Viruses with Adjuvant

Ability to optimize the immunogenicity of the vaccine is now also a current topic of high priority in the field of applied immunology, especially as a means of controlling HIV infection (Sasaki et al, 1998). One approach to optimize the response elicited by the immune system against the vaccine is to couple it with an adjuvant, which can increase the type, strength and durability of immune responses evoked by a vaccine (Velin et al, 1998). Some vaccine antigen/adjuvant combinations can induce cell-mediated immune responses in animals, even if the vaccine antigen by itself does not (Velin et al, 1998).

Currently, only one adjuvant—alum, first discovered in 1926—is incorporated into vaccines licensed for human use by the U.S. FDA. An adjuvant may work well with one experimental vaccine but not another, therefore, the FDA licenses the vaccine formulation, or the antigen-adjuvant combination, rather than the adjuvant alone (Velin et al, 1998). Alum primarily increases the strength of antibody responses generated by the vaccine antigen. Because of alum's limited activity, other adjuvants now being evaluated in animal models and human studies may be better suited for the newer candidate HIV vaccines.

A study by Sasaki research the effect of Ubenimex (UBX), an anti-cancer immunomodulator, as an adjuvant on a DNA

AIDS vaccine which was developed and examined in a murine (mice) model. With the titers of IgG in the sera collected in the mice being 2-5 times higher than those inoculated without the use of UBX as an adjuvant, along with other evidence including stronger cytotoxic T lymphocyte activity, raised IL-2 and interferon-gamma levels, it is clear to see that the use of UBX as an immunologic adjuvant for DNA vaccination against HIV-1 may be very suitable for clinical use because of its lack of antigenicity and low toxicity (Sasaki et al, 1998).

Whole Killed or Inactivated Viral Vaccines

Whole-killed or inactivated viral vaccines have been traditionally very effective in preventing disease (e.g., inactivated polio, hepatitis A, and influenza). However, failed animal challenge experiments with inactivated HIV vaccines, as well as safety concerns over the possibility of incomplete inactivation or DNA integration in the host, have significantly impeded progress with this approach (Heyward et al, 1998). Overall, there was not much data focussing on this approach to the vaccine to be found.

Live Vector Vaccine

A live bacteria or virus that is harmless to humans and is used to transport a gene that makes HIV proteins is now being explored as a possible way to vaccinate against

HIV (Heyward et al, 1998). Current vectors that are being explored include vaccinia virus, canarypox, adenovirus, salmonella, bacille Calmette-Gueurin, and poliovirus (Heyward et al, 1998). The primary focus on live recombinant vectors concentrates on using poxvirus (canarypox or vaccinia). These vaccines have the ability to produce both neutralizing antibodies and CD8+ CTLs in low to moderate levels in approximately 30 to 40 percent of subjects (Heyward et al, 1998).

A study out of the Division of Infectious Diseases and Immunology at Saint Louis University School of Medicine determined that the live canarypox vector was safe, stimulating cytotoxic T-cells and priming for a vigorous neutralizing antibody response upon boosting with the gp120 subunit vaccine (Belshe et al, 1998). The authors concluded that used as a vector vaccine, the vaccine might have the potential for producing long term protection against HIV infection (Belshe et al, 1998).

Human Clinical Trials

Phase I/II Safety and Immunogenicity Trials

Since 1987, more than 21 HIV-1 preventive candidate vaccines have been tested in Phase I trials to assess their safety and immunogenicity. Although the immune correlates of protection against HIV infection are not known, the

first generation of candidate vaccines were aimed at inducing neutralizing antibodies, and most of them have been based on subunit recombinant envelope concept including gp120 and gp160 envelope glycoproteins. These first vaccines have been replaced by new candidates including DNA, live-attenuated virus, and live vectors, which are now entering Phase I/II clinical trials. These possible routes for vaccination, which were reviewed above, will have to move through the most important Phase III trials before they can be used in the human population.

Phase III Efficacy Trials

Although no country at this time has decided to proceed with a Phase III trial to determine the efficacy of candidate vaccines, several developed and developing countries are now actively preparing populations and research infrastructures for such trials (Esparza et al, 1991). These preparations include characterization of potential trail populations, and the establishment of cohorts of HIV-negative volunteers for the determination of HIV incidence and their willingness to participate in trials (Heyward, Osmanov, Saba, et at 1994);social-behavioral studies to ensure proper educational counseling, and informed consent (Chesney et al, 1995);virological studies to characterize incidence of HIV-1 strains in trial

populations and the development of guidelines to ensure the highest scientific and ethical standards in the conduct of trials, with the appropriate participation of the community.

Phase III trials will require extensive international collaboration and coordination, with the developing countries playing a major role in these trials, since some of the highest HIV incidence rates are found in developing countries, and conducting trials among these populations would reduce the sample size and duration of the trial(Cohen, 1995). More importantly, since over 90% of the incidence of HIV infection occurs in the third world countries, they would eventually benefit the most from an effective HIV vaccine (Cohen, 1995). With the amount of genetic variability seen in the HIV-1 subtype, coupled with various co-factors and routes of transmission in different geographic areas, multiple efficacy trials need to be conducted at the same time in different parts of the world. In lieu of all of these hurdles necessary to move on to the Phase III trials, decisions to initiate the trials will be difficult. However, these decisions must be based not only on an analysis of scientific data, but also on important public health considerations (severity of the epidemic), as well the feasibility of conducting large-scale trials,

including: the ability to recruit and follow a large number of volunteers, and the political and community support to ensure successful implementation of the trial.

Phase III trials will be an enormous scientific and social challenge, both expensive and time consuming. Some have raised the issue that potential vaccine efficacy trial 'side effects' may occur, such as false expectations by the community that an effective vaccine will soon be available, with could interfere with other prevention strategies, such as protected sex, that are now are the only way to avoid the disease. Most importantly, serious rare adverse events such as antibody-dependent enhancement of infectivity may occur when large numbers of volunteers are vaccinated, and that a poorly designed or conducted trial, or even a trial with a 'no efficacy' result, may create an atmosphere of pessimism or rejection of further efforts to curb the HIV epidemic through vaccine development.

One study conducted at the Department of Psychology, at Indiana University Purdue University at Indianapolis (IUPUI) concluded that there a number of psychosocial barriers to the HIV vaccine acceptance, and it cannot be assumed that there will be a universal acceptance for all people (Liau et al, 1998). The team of researchers headed by Liau cited the possible risk of infection as being the

most critical obstacle in participating in a clinical trial of the candidate vaccine.

Therefore, in order to initiate a Phase III clinical trial, a careful risk benefit analysis must be conducted. Testing available candidate vaccine(s) must be weighed against the severity of the epidemic and the consequences of waiting for more data or 'better' candidate vaccines in the future (Mariner, 1990).

Conclusion

Discussions on strategies of how to develop HIV vaccines are often hampered by the confrontation of two truisms: One states that the more information we obtain from basic research, the better off are we to develop more effective HIV vaccines. The other argues that laboratory research alone will never be a substitute for large-scale clinical trials to obtain definitive information on vaccine efficacy (Letvin,1998). A passionate and uncompromising defense of either position will not help those people who have to take the practical decisions, nor will this effectively promote HIV vaccine development in general. Thus, a sensible strategy is to accept the uncertainties of proceeding with efficacy trials of available products which have met previously defined minimal requirements and at the same time continue basic research to obtain additional

information on the nature of protective immune responses in humans, some of which would likely be derived from the efficacy trials themselves (Haynes, 1993).

From a practical point of view, it would be important to address three questions: (1) What type of additional information is necessary to proceed to efficacy trials with the greatest likelihood of success? (2) How realistic are the expectations that relevant information will be obtained from additional laboratory, animal protection or natural history studies in the absence of efficacy trials? (3) From the candidate vaccines which have entered Phase I/II trials, are there products that meet minimal conditions to proceed to Phase III trials?

Answering the above questions is not easy. Since natural immune responses to HIV are complex (including both humoral and cellular responses) and obviously not very efficient, focusing laboratory research on limited aspects of the human immune response to HIV infection and disease may lead in false directions. Likewise, great uncertainties remain concerning the relevance of animal models as predictors of vaccine efficacy in humans.

Several candidate vaccines, based on different concepts, are at different stages in the HIV vaccine development 'pipeline' (Levy, 1995). Candidate vaccines

based on the subunit recombinant envelope concept and produced in mammalian cells, have been shown to protect chimpanzees from HIV-1 infection, and to be safe and reasonably immunogenic in humans, inducing neutralizing antibodies. A second generation of candidate vaccines, which are based on live vectors expressing the envelope and other HIV-1 genes, and which are capable of inducing CTLs, are beginning to be evaluated in human trials. Newer generations of candidate vaccines now being mostly explored in animal experiments are using combinations of subunit recombinant proteins or live-vectored vaccines with other immunogens or are based on more novel approaches, including nucleic acid immunization and perhaps whole-inactivated or live-attenuated vaccines.

With our present state of knowledge, it is not possible for laboratory assays to accurately predict which vaccine concept, or concepts, will induce protection against HIV infection in humans. Unless major advances are made in our understanding of the nature of protective immune responses to HIV-1 in humans, that information will only be obtained through the conduct of Phase III field efficacy trials. However, in view of the rate of progression of the HIV pandemic, especially in developing countries, it would not be ethical to wait in the hope that

such advances will occur soon, thus postponing trials with candidate vaccines (Heyward, Osmanov, Saba, et al, 1994). In fact these trials, conducted in parallel or sequentially, may represent our best chance to enhance our basic knowledge of the nature of protective immune responses to HIV infection.

Thus, in order to avoid the unacceptable alternative of perpetual uncertainty, or to delay the development of a much-needed vaccine, there is no other choice but to effectively integrate further basic research with the initiation of large-scale field efficacy trials in the process of HIV vaccine development. These Phase III trials will present unique opportunities to: (1) establish if different vaccine concepts can induce protection in humans; (2) validate the primate models presently being used in HIV vaccine research; (3) obtain information on immune correlates of vaccine-induced protection; (4) explore the significance of viral genetic variability in relation to vaccine-induced protection; (5) evaluate different end-points for vaccine efficacy (prevention of infection, establishment of chronic infection, or disease); and (6) generate additional data on vaccine safety (Heyward et al, 1997).

The development of an HIV vaccine will be a long and difficult process. Multiple efficacy trials and case-control studies will ultimately be required before a safe, effective and affordable vaccine is available for widespread public health use. With more than 6000 new infections occurring every day worldwide, there is urgency to proceed.

.Works Cited

1. Andre S; Seed B; Eberle J; Schraut W; Bultmann A; Haas J "Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage." *Journal of Virology* 1998 Feb;72(2):1497-503

2. Bagarazzi ML; Boyer JD; Javadian MA; Chattergoon M; Dang K; Kim G; Shah J; Wang B; Weiner DB; "Safety and immunogenicity of intramuscular and intravaginal delivery of HIV-1 DNA constructs to infant chimpanzees." *Journal of Medical Primatology* 1997 Feb-Apr;26(1-2):27-33

3. Behforouz, Nancy Dr. Thesis Advisor's Advice. Professor of Immunology at Ball State University, Muncie IN.

4. Belshe RB; Gorse GJ; Mulligan MJ; Evans TG; Keefer MC; Excler JL; Duliege AM; Tartaglia J; Cox WI; McNamara J; Hwang KL; Bradney A; Montefiori D; Weinhold KJ "Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers." *AIDS* 1998 Dec 24;12(18):2407-15

5. Berman DW;"Development of bivalent rgp120 vaccines to prevent HIV type 1 infection."*AIDS* Research of Human Retroviruses 1998 Oct;14 Suppl 3:S277-89

6. Chesney MA, Lurie P, Coates TJ: "Strategies for addressing the social and behavioural challenges of prophylactic HIV vaccine trials." *Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology* 1995, 9:30 - 35

7. Cohen J; "Thailand weighs AIDS vaccine tests." *Science* 1995, 270:904 - 907

8. Connor RI; Korber BT; Graham BS; Hahn BH; Ho DD; Walker BD; Neumann AU; Vermund SH; Mestecky J; Jackson S; Fenamore E; Cao Y; Gao F; Kalams S; Kunstman KJ; McDonald D; McWilliams N; Trkola A; Moore JP; Wolinsky SM; "Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines." *Journal of Virology* 1998 Feb;72(2):1552-76

9. Des Jarlais DC; Vanichseni S; Marmor M; Buavirat A; Titus S; Raktham S; Friedmann P; Kitayaporn D; Wolfe H;

Friedman SR; Mastro TD; "Why I am not infected with HIV": implications for long-term HIV risk reduction and HIV vaccine trials." *Journal Acquired Immune Deficiency Syndrome and Human Retrovirology* 1997 Dec 15;16(5):393-9

10. Desrosiers RC; "HIV with multiple gene deletions as a live attenuated vaccine for AIDS." *AIDS Res Hum Retroviruses* 1992;8:411-421.

11. Esparza J; Osmanov S; Kallings LO; Wigzell H; "Planning for HIV vaccine trials: the World Health Organization perspective." *AIDS* 1991, 5(suppl 2):S159 - S163

12. Graham BS, Wright PF; "Candidate AIDS vaccines." *New England Journal of Medicine* 1995;333:1331-1339

13. Haynes BF; "Scientific and social issues of Human Immunonodeficiency Virus vaccine development." *Science* 1993, 260:1279 - 1286.

14. Heyward WL; MacQueen KM; Goldenthal KL; "HIV vaccine development and evaluation: realistic expectations." *AIDS* Res Hum Retroviruses 1998 Oct;14 Suppl 3:S205-10

15. Heyward WL; MacQueen KM; Jaffe HW; "Obstacles and progress toward development of a preventive HIV vaccinewith 40 phase I trials, only one for phase 2, and none for phase 3, the term "HIV vaccine development pipeline" may be a misnomer."*Journal for the Center for Disease Control* August, 1997

16. Heyward WL; Osmanov S; Saba J; *et al;* "Preparation for Phase III HIV vaccine efficacy trials: methods for the determination of HIV incidence." *AIDS* 1994,8:1285 - 1291.

17. Hilleman MR; "A simplified vaccinologists' vaccinology and the pursuit of a vaccine against AIDS." *Vaccine* 1998 May;16(8):778-93

18. Hulskotte EG; Geretti AM; Osterhaus AD; "Towards an HIV-1 vaccine: lessons from studies in macaque models." *Vaccine* 1998 May-Jun;16(9-10):904-15

19. Johnston MI; "HIV vaccines: problems and prospects." International AIDS Vaccine Initiative, Washington, DC,USA. Hosp Pract (Off Ed) 1997 May 15;32(5):125-8, 131-40

20. Josefson D; "Approval given for trials of AIDS vaccine." *British Medical Journal* 1998 Jun 13;316:1769

21. Lambert JS; McNamara J; Katz SL; Fenton T; Kang M; VanCott TC; Livingston R; Hawkins E; Moye J Jr; Borkowsky W; Johnson D; Yogev R; Duliege AM; Francis D; Gershon A; Wara D; Martin N; Levin M; McSherry G; Smith G "Safety and immunogenicity of HIV recombinant envelope vaccines in HIV- infected infants and children." *Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology* 1998 Dec 15;19(5):451-61

22. Letvin NL; "Progress in the development of an HIV-1 vaccine." *Science* 1998 Jun 19;280(5371):1875-80

23. Levy JA; "HIV research: a need to focus on the right target." *Lancet* 1995, 345:1619 - 1621.

24. Liau A; Zimet GD; Fortenberry JD; "Attitudes about human immunodeficiency virus immunization: the influence of health beliefs and vaccine characteristics." *Sexually Transmitted Diseases* 1998 Feb;25(2):76-81

25. Mariner WK; "The ethical conduct of clinical trials of HIV vaccines." *Evaluation Review* 1990;14:538-564.

26. McCutchan FE; Sanders-Buell E; Salminen MO; Carr JK; Sheppard WH; "Diversity of the human immunodeficiency virus type 1 envelope glycoprotein in San Francisco Men's Health Study participants." *AIDS* Research of Human Retroviruses 1998 Mar 1;14(4):329-37

27. Mortara L; Letourneur F; Gras-Masse H; Venet A; Guillet JG; Bourgault-Villada I; "Selection of virus variants and emergence of virus escape mutants after immunization with an epitope vaccine." *Journal of Virology* 1998 Feb;72(2):1403-10

28. No Author Given; "HIV vaccine development." *BMJ* 1998 Jul 18;317:163. http//:www.bmj.com

29. Osborn JE; "The rocky road to an AIDS vaccine." *Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology* 1995, 9:26 - 29.

30. Pantaleo G, Menzo S, Vaccarezza M, *et al*; "Studies in subjects with long-term nonprogressive human immunodeficiency virus infection." *New England Journal of Medicine* 1995, 332:209 - 216.

31. Perlman D; "AIDS vaccine at least 10 years away, experts say several good leads, but none ready for large human trials" *Chronicle Science Editor* Wednesday, May 13, 1998

32. Ruprecht RM; Baba TW; Greene MF; Almond N; Cranage M; Stott J; "Attenuated vaccines for AIDS?" *Lancet* 1995, 346:177 - 179

33. Sasaki S; Hamajima K; Fukushima J; Ihata A; Ishii N; Gorai I; Hirahara F; Mohri H; Okuda K "Comparison of intranasal and intramuscular immunization against human immunodeficiency virus type 1 with a DNA-monophosphoryl lipid A adjuvant vaccine." *Infectious Immununology* 1998 Feb;66(2):823-6

34. Tung FY; Rinaldo CR Jr; Montelaro RC; "Replication-defective HIV as a vaccine candidate." *AIDS* Res Hum Retroviruses 1998 Sep 20;14(14):1247-52

35. Ugen KE; Nyland SB; Boyer JD; Vidal C; Lera L; Rasheid S; Chattergoon M; Bagarazzi ML; Ciccarelli R; Higgins T; Baine Y; Ginsberg R; Macgregor RR; Weiner DB "DNA vaccination with HIV-1 expressing constructs elicits immune responses in humans." *Vaccine* 1998 Nov;16(19):1818-21

36. Velin D; Hopkins S; Kraehenbuhl J "Delivery systems and adjuvants for vaccination against HIV." *Pathobiology* 1998;66(3-4):170-5

37. World Health Organization; "Scientific and public health rationale for HIV vaccine efficacy trials." *AIDS* 1995;9:WHO1-WHO4.

38. Wyatt R; Sodroski J; "The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens." *Science* 1998 Jun 19;280(5371):1884-8